Effects of 1-Methylcyclopropene on Quality and Antioxidant Capacity of in Vitro Digests from Ziziphus Mauritiana

Songshan Qiu*[1], Cuicui Jiang[2], Rujin Zhou[3]

*[1, 2, 3]School of Chemistry and Life Science, Guangdong Institute of Petrochemical Technology
Maoming, China

*[1]tustqss@163.com

Abstract

The effects of 1-methylcyclopropene (1-MCP) on preservation quality and antioxidant bioavailability of *Ziziphus Mauritiana* were examined during the period of shelf life. Fruit were exposed to 0.5 (μLL^{-1}) of 1-MCP for 24 h at room temperature, following storage, fruit were held up to 8 days and assessed for quality, membrane permeability, malondialdehyde (MDA), and using an *in vitro* digestion system that simulated the physiochemical changes occurring in the upper gastrointestinal tract. The differences in fruit firmness, soluble solid concentration (SSC), and membrane permeability with 1-MCP treatment were manifest during shelf life period. MDA, an indicator of lipid peroxidation caused by reactive oxygen species, increased during shelf life and the contents were significant lower in fruit treated with 1-MCP than control samples. The results indicated that 1-MCP is an effective tool for quality improvement and extension of shelf life in jujubes, and showed that the bioaccessible antioxidant capacity of tissues is tightly related to the quality of fruits and the simulated digestion appears to be an efficient tool to study the bioaccessible antioxidant from fruits and vegetables.

Keywords

Ziziphus Mauritiana, Antioxidant, 1-methylcyclopropene

Introduction

Bioaccessibility, an important factor in nutrition research due to its variation among different foods, foods matrices and gastrointestinal conditions, can be defined as the proportion of a nutrient that can be utilized for normal physiological functions. The main component of bioaccessibility refers to the digestion and absorption of nutrients in the gut, which is the main rate-limiting factor. *In vitro* models based on human physiolsongshanogy have been developed as simple, inexpensive and reproducible tools to study digestive stability, intestinal transport and metabolism and to predict the bioavailability of different food components (i.e., ascorbic acid, carotenoids, glucosinolates) [Agata et al. (2009)]. Most of the *in vitro* digestion models simulate in a simplified manner the digestion processes in stomach and small intestine, in order to enable investigation of the bioaccessibility of compounds from their matrix during transit in the gastrointestinal tract. The use of *in vitro* digestion models may help provide more insight into the bioavailability of antioxidants from the food matrix. Fruits and vegetables are the major sources of biologically active compounds (i.e., phytochemicals), and an increased consumption is recommended. Evidences suggest that only 15-30% of the antioxidant in orange juice is extractable by digestive fluid [Chong et al. (2006)]. It is now clear that the ultimate antioxidant potential of phytochemicals and their resulting *in vivo* bioactivity is dependent on their absorption, metabolism within the body after digestion. Assessment of true bioaccessibility of any class of phytochemicals requires data concerning their absorption, metabolism, tissue and organ distribution and excretion [Dong et al. (2012)]. Therefore, it is important to establish the relationship of total antioxidant content to bioavailable content in the context of treatment applications that are intended to enhance quality retention in fresh fruit and vegetable products.

Zizyphus species are commonly used in folklore medicine for the treatment of various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anaemia, and insomnia [George et al. (2005)]. *Ziziphus Mauritiana*, a native fruit of China, has been commonly used as a crude drug in traditional

Chinese medicine and also commonly used as food, food additive and flavourant for thousands of years [Gil-Izquierdo et al. (2001)]. Much of the annual jujube production has been consumed in fresh and dried forms, and currently, there have numerous studies, experimental works focused on the preservation and processing of Chinese jujube to enhance postharvest quality [Hodges et al. (1999), Khan et al. (2011)]. However, little information on the regulation of ripening of various cultivars of *Ziziphus Mauritiana* is available. Ethylene plays an important role in ripening and senescence of various fruits, vegetables and flowers [Li et al. (2007)]. Of the ethylene action inhibitors available, 1-methylcyclopropene (1-MCP) has proven most effective at suppressing ripening and adverse ethylene responses in numerous plant tissues [Miller et al. (1981), Re et al. (1999)]. In this study, we investigated the influence of 1-MCP treatment on maintenance of quality, functional properties and membrane lipid peroxidation of *Ziziphus Mauri tiana* on the shelf life. An *in vitro* digestion procedure in the stomach and small intestine was mimicked respectively and the bioaccessible antioxidant capacity and reducing power of the fractions was determined on digestates of gastric and pancreatic digestion protocols.

Materials and Method

Fruit Materials and Reagents

Ziziphus Mauritiana was harvested from *Ziziphus Mauritiana Lamk*. cv. Maoming city, Guangdong province (China) and then selected based on their uniformity of shape, size and color. Any physical damaged or diseased ones were discarded. Fruits were placed in 10 L container and exposed to 0.5 (μL L^{-1}) 1-MCP generated by SmartFresh (Rohm and Haas Co., Italy) at room temperature and 80-90% RH for 24 h as described by [Shanmugam et al. (2011)], followed by the removal from the container after 1-MCP treatment, and subsequently held at 0°C for one month and different days at room temperature to simulate a shelf life. For each treatment, triplication was conducted. The qualities of fruits were evaluated during shelf life period followed by 0, 2, 4, 6 and 8 days at room temperature (22°C) and then the samples were cored, sliced and the slices of cortex tissue immediately flash frozen in liquid nitrogen. Tissue was stored at −80°C until use. Firmness, soluble solids concentration (SSC), MDA content, Membrane permeability and antioxidant were determined individually during shelf life. Each sample analysis was repeated three times. Unless otherwise stated, all solvents, salts and acids (AR) were supplied by China Oleochemicals Company.

Fruit Quality Assessments

Firmness of fruit was measured using a GY-1 firmness tester fitted with a 3.5 mm diameter head (Hangzhou Top Instrument Inc, China), after skin removal at room temperature. Ten fruits were randomly measured for each treatment during shelf life and data were expressed as N/m^2. Soluble solid concentration (SSC) was recorded with a hand refractometer (Tongfang Inc, Shanghai, China) in juice. MDA content was assayed with the thiobarbituric acid (TBA) reaction, according to the reported method [Singh & Rajini (2004)]. Briefly, about 0.2 g of pulp was dissolved in 10 ml of 10% (w/v) trichloroacetic acid; and the mixture was centrifuged at 4000 g for 10 min. After incubation of 2 ml of the supernatant with 2 ml with the weight of 6.7 g L^{-1} TBA for 30 min at 95ºC, the mixture was quickly cooled in an ice bath and further centrifuged at 4000 g for 10 min. The absorbance of the supernatant was read at 532, 450 and 600 nm, respectively, with the 756-PC UV/VIS spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China) and the results were expressed as μmol g^{-1} fresh weight (f.w.).

$$\text{MDA content } (\mu\text{mol } g^{-1}\text{f.w.}) = 6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}. \quad (1)$$

Membrane permeability, expressed by relative leakage rate, was determined following the methods of Zhang et al. [Singh & Rajini (2004)] and Wahid and Shabbir [Sisler & Serek (1997)] with minor modification. Fruits were sliced into small discs with 0.05 cm thickness and washed three times with deionized water to remove surface-adhered electrolytes. After drying with filter paper, 10 discs were placed in closed vials containing 20 ml deionized water in closed vials containing 30 ml of deionized water and shaken at 25ºC on a rotary shaker for 30 min, subsequently electrical conductivity of the solution was determined, using a conductivity meter (model DDS-307, Shanghai Precision & Scientific Instrument Co., Ltd., China). The vials with solution were then boiled for 10 min, quickly cooled and the total electrical conductivity was obtained. Relative leakage rate was expressed as percent of total electrolytes.

In Vitro Digestion Procedure

An *in vitro* method was used to evaluate bioaccessibility of antioxidants and the protocols were based on those developed by Miller et al. [Sisler & Serek (2003)] and George et al. [Toivonen & Lu (2005)]. The method consisted of two sequential steps: 1) an initial pepsin digestion at low pH for 30 min at 37°C to simulate gastric conditions followed by, 2) a pancreatin digestion at high pH for 1 h at 37°C to simulate small intestine conditions. A 5 g aliquot of frozen tissue was first thawed and then made into slurry in 10 mL saline solution (140 mM NaCl plus 5 mM KCl). An 8 mL aliquot of the slurry was added to 8 mL of simulated gastric fluid (SGF, 3.2 (g L^{-1}) pepsin, 2.0 (g L^{-1}) NaCl and 7 mL 12 (mol L^{-1}) HCl, pH 1.2) and the mixture was incubated in a 37°C shaking water bath set at 60 rpm for 30 min. The gastric digestate was then pH adjusted with the addition of 0.4 mL 0.2 (mol L^{-1}) NaOH. The pH adjusted digestate was then added to 16 mL simulated intestinal fluid (SIF, 10.0 (g L^{-1}) pancreatin and 6.8 (g L^{-1}) KH_2PO_4, pH 6.8) and incubated in a 37°C shaking water bath for 60 min. The final digestate was centrifuged at 5000 *g* at 20°C for 30 min in a centrifuge 5804 R (Eppendorf China Ltd., Beijing, China). A 3.5 mL aliquot of the resultant supernatant was transferred to the top well of a centrifugal size exclusion filter device (Amicon Ultra-4. 10k molecular weight cut off, Millipore Corp., Billerica, MA) and centrifuged at 5000 *g* in a centrifuge 5804 R for 30 min at 20°C. An aliquot of the resultant filtrate analyzed for antioxidant capacity represented the total antioxidant capacity of the digestate.

Determination of Reducing Power

The reducing power of the digestate was quantified by the method described earlier by [Vandenbussche et al. (2012)] with minor modifications. To a 1 mL aliquot of the digestate, 1 mL of 0.2 M phosphate buffer with pH value of 6.6 and 1 mL of 1% $K_3Fe(CN)_6$ were added. The mixture was incubated at 50°C for 20 min. The reaction was terminated by adding TCA solution (10%) and the mixture was centrifuged at 2000 g for 10 min. One mL of the supernatant was taken to which 1 mL of distilled water and 0.2 mL of 0.1% $FeCl_3$ solution was added. The absorbance was measured at 700 nm using 756-PC UV/VIS spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China). Samples were analyzed in two runs, for each of which, three determinations were made and all values were averaged. Increment in absorbance of the reaction mixture indicated the increased reducing power of samples.

ABTS Free Radical Scavenging Assay

The ABTS free radical scavenging ability of the digestate was tested by a modified method as described by [Wahid & Shabbir (2005)]. ABTS radical cation (ABTS) was produced by reaction of 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to be kept in the dark at room temperature for 12-16 h before use. The ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 25 μL of sample or trolox standard to 2 mL of diluted ABTS solution, absorbance at 734 nm on the 756-PC UV/VIS spectrophotometer was measured at exactly 6 min. The ABTS radical scavenging ability of sample was also expressed as trolox equivalent antioxidant capacity (TEAC) (μmol /g f.w.)

Statistical Analysis

All experiments were carried out three times for each condition tested, and for each experiment the analysis was performed in duplicate. All data were expressed as means ± standard errors of three replicates on the figures and tables. The significance of 1-MCP treatment compare with control was determined by analysis of variance using orthogonal contrast statements in a general linear models procedure (PROC GLM; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Effect of 1-MCP Treated on Fruit Quality

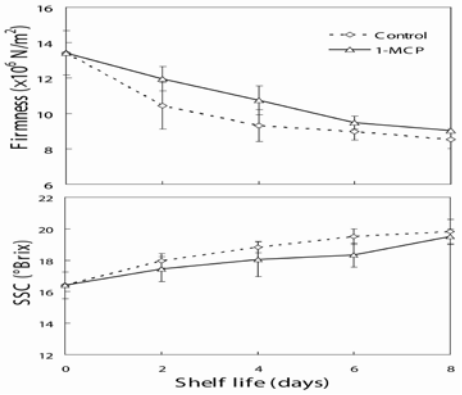

FIGURE 1 DIFFERENCE IN FIRMNESS AND SOLUBLE SOLID CONCENTRATION (SSC) OF 1-MCP- AND NON- TREATED (CONTROL) SAMPLE WHEN STORED AT ROOM TEMPERATURE

FOR 8 DAYS SHELF LIFE.

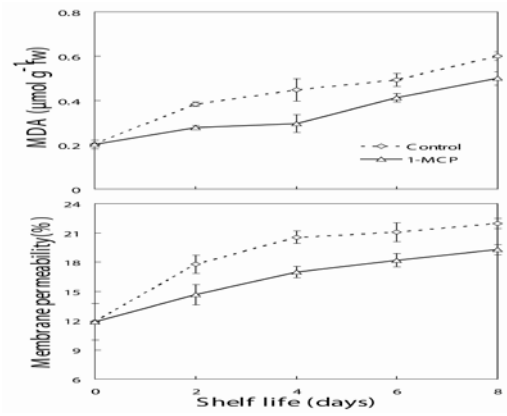

FIGURE 2 DIFFERENCE IN MDA CONTENT AND MEMBRANE PERMEABILITY OF 1-MCP- AND NON- TREATED (CONTROL) SAMPLE WHEN STORED AT ROOM TEMPERATURE FOR 8 DAYS SHELF LIFE.

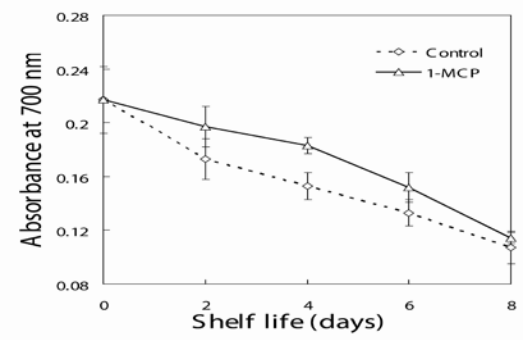

FIGURE 3 RADICAL SCAVENGING ACTIVITY OF THE DIGESTATE OF 1-MCP- AND NON- TREATED (CONTROL) SAMPLE DURING SHELF LIFE.

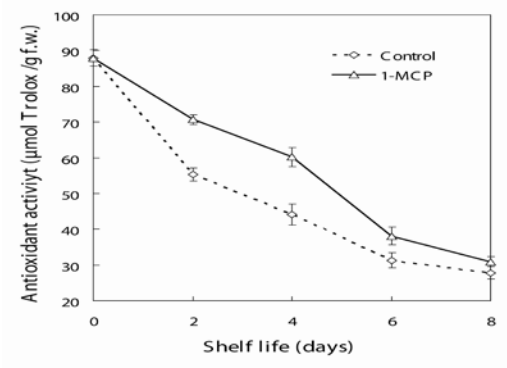

FIGURE 4 COMPARISON OF THE ANTIOXIDANT CAPACITY OF THE DIGESTATE OF 1-MCP- AND NON- TREATED (CONTROL) SAMPLE MEASURED AS TROLOX EQUIVALENTS (μMOL TROLOX G^{-1}F.W.) BY ABTS ASSAYS DURING SHELF LIFE.

According to the research by [18], fruit softening is due to the degradation of cell wall components, mainly pectin's, by the action of specific enzymes, such as polygalacturonase. As shown in Fig. 1, firmness of control fruit decreased rapidly within 8 days of storage at room temperature and reached a value of 8.5 ($\times 10^6$ N/m^2). Firmness was significantly higher in fruit treated with 1-MCP than that in the control fruit during shelf life period (Figure 1). The results of softening showed that 1-MCP treated had a significant delay of firmness and had a beneficial effect on firmness retention. Soluble solids concentration of jujube increased with time during shelf life period as expected. The results also showed that there were significant differences of firmness and SSC between control and 1-MCP treated fruit during shelf-life period (Figure 1). Thus MDA is usually as an indicator of the degree of plant oxidative stress. In the present study, there was a continuously increase in MDA content, both in control and 1-MCP treated fruit (Figure 2), yet the application of 1-MCP to fruit significantly delayed the increase of MDA at the level of P less than 0.05. After 8 day storage, the MDA content of jujube with 1-MCP treated (0.5 ± 0.03 μmol g^{-1}f.w.) was significantly lower than that of the control ($P < 0.05$). Significant lipid peroxidation in control jujubes during shelf life was shown by the accumulation of MDA. This result is in agreement with the report that different treatment may significantly change the MDA contents of jujube.

Membrane permeability changes during storage were assessed by determining the intensity of ion (electrolyte) leakage. This parameter was included in order to have more information on membrane stability and thereby on the relative ion content in the apoplastic space. In the present study, the change in membrane permeability shared similar trends with MDA content. Membrane permeability, as an indicator of membrane integrity, gradually increased during shelf life period. As shown in Figure 2, the rapid increase of membrane permeability in the control fruit was much higher than that in the 1-MCP treated fruit, especially beyond 8 days of storage ($P < 0.05$). However, 1-MCP-treated fruit had significantly lower relative leakage rates than the control fruit (Figure 2), indicating that a higher membrane integrity was maintained.

Bioaccessible Antioxidant Capacity and Reducing Power of the Digestate

The reducing power assay measures the electron-donating ability of antioxidants using the potassium ferricyanide reduction method. Different studies have indicated that antioxidant activity and reducing power are related. The digestate fractions of *Ziziphus Mauritiana* between 1-MCP- and non-treated exhibited reducing power and the results are presented in Figure 3. The reducing power was significantly higher in fruit treated with 1-MCP than that in the control fruit during shelf life storage. A similar observation has been

reported by on wheat germ protein hydrolysate. It seems that the reducing power of the digestate may be due to the presence of phenolic compounds or flavonoids. In ABTS radical cation scavenging method, the antioxidant capacity of the digestate *in vitro* digestion expressed as Trolox equivalents (μmol Trolox/g f.w.) was shown in Figure 4. There were significant differences in ABTS radical cation scavenging activity between 1-MCP treated and control samples. As shown in Figure 4, the values of antioxidant activity with 1-MCP treatment were 70.7 to 60.2 μmol Trolox /g f.w. in the second and fourth days, respectively, compared with 55.3 to 44.1 (μmol Trolox /g f.w.) in control. The results indicated that the antioxidant capacity of sample was largely affected by 1-MCP treatment during shelf life period.

Conclusions

The 1-MCP treatment maintained the firmness, soluble solids concentration and membrane permeability of *Ziziphus Mauritiana* compared with the untreated, and the effect was particularly significant during the 8 days shelf life. MDA contents were lower in fruit treated with 1-MCP than that in control samples within 8 days. Our results indicated that 1-MCP treatment could improve fruit quality and inhibit membrane lipid peroxidation and reduce the formation of MDA during shelf life. *In vitro,* simulated gastrointestinal digestion studies showed that the bioaccessibility of antioxidants were best maintained 1-MCP treated fruit during shelf life. Fractionation of simulated gastrointestinal model digestate of the fruit provided a new insight as to the relative importance of phytochemicals in relation to bioaccessible antioxidant capacity.

ACKNOWLEDGEMENTS

This research work was financially supported by the Science and Technology Plan Projects of Guangdong province (No. 2011B010100046) and by the cooperation project in industry, education and research of Guangdong province and Ministry of Education of P. R. China.

REFERENCES

Agata, M.P., Fabiano, C., Ammar, B. and Alessandra, B. 2009. Flavonoids of Zizyphus jujuba L. and Zizyphus spina-christi (L.) Willd (Rhamnaceae) fruits. Food Chemistry, 112:858-862.

Chong, C., Chen, K.S., Xu, W.P., Zhang, W.S. Li, X. and Ian, F. 2006. Effect of 1-MCP on postharvest quality of loquat fruit. Postharvest Biology and Technology, 2:155-162.

Dong, Z.Y., Wang, G.L., Liu, X., Liu, J., Zhu, D.Z. and Ling, C.Q. 2012. Treatment of cholecystitis with Chinese herbal medicines: a systematic review of the literature. World Journal of Gastroenterology, 14:1689-1694.

George, S., Brat, P., Alter, P. and Amiot, M.J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. Journal of Agricultural and Food Chemistry, 53:1370-1373.

Gil-Izquierdo, A., Gil, M.I., Ferreres, F. and Tomás-Barberán, F.A. 2001. In vitro availability of flavonoids and other phenolics in orange juice. Journal of Agricultural and Food Chemistry, 2:1035-1041.

Hodges, D.M., Delong, J.M., Forney, C.F. and Prange, R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta, 207:604-611.

Khan, S.H., Ahmad, F., Ahmad, N., Flynn, D.C. and Kumar, R. 2011. Protein-protein interactions: principles, techniques, and their potential role in new drug development. Journal of biomolecular structure & dynamics, 6, 929-938.

Li, J., Fan, L.P., Ding, S.D. and Ding, X.L. 2007. Nutritional composition of five cultivars of Chinese jujube. Food Chemistry, 103:454-460.

Miller, D.D., Schricker, B.R., Rasmussen, R.R. and Vancampen, D. 1981. An in vitro method for estimation of iron availability from meals. American Journal of Clinical Nutrition, 34, 2248-2256.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Riceevans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26:1231-1237.

Shanmugam, S., Park, J.H., Kim, KS, Piao, Z.Z., Yong, C.S., Choi, H.G. and Woo, J.S. 2011. International journal of pharmaceutics, 1-2:99-105.

Singh, N, and Rajini, PS. 2004. Free radical scavenging activity of an aqueous extract of potato peel. Food Chemistry, 85:611-616.

Sisler, E. C. and Serek, M. 1997. Inhibition of ethylene

responses in plants at the receptor level: recent developments. Plant Physiology, 100:577-582.

Sisler, E. C. and Serek, M. 2003. Compounds interacting with the ethylene receptor in plants. Plant Biology, 5:473-480.

Toivonen, P. M. A. and Lu, C. 2005. Studies on elevated temperature, short-term holding of 'Sunrise' summer apples using 1-MCP to maintain quality. Journal of Horticultural Science and Biotechnology, 80:439-446.

Vandenbussche, F., Vaseva, I., Vissenberg, K. and Van, Der Straeten D. 2012. Ethylene in vegetative development: a tale with a riddle. New Phytologist, 4:895-909.

Wahid, A. and Shabbir, A. 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. Plant Growth Regulation, 46:133-141

Wang, Y., Tang, F., Xia, J., Xu T., Wang, J., Azhati, R. and Zheng, X.D. 2011. A combination of marine yeast and food additive enhances preventive effects on postharvest decay of jujubes (Zizyphus jujuba). Food Chemistry, 3:835-840.